Triglyceride(TG) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Microplate reader/Spectrophotometer

Catalog Number: NA0577

Size:100T/96S

Components:

Reagent I: Self-provided reagent, add 80 mL of n-heptane and 80 mL of isopropyl alcohol to an empty glass bottle. Seal and mix well, storage at 4℃.

Reagent II: 3 mL×1 bottle. Storage at 4℃.

Reagent III: 10 mL×1 bottle. Storage at 4℃.

Reagent IV: 3 mL×1 bottle. Storage at 4℃, protected from light.

Reagent V: 10 mL×1 bottle. Storage at 4℃, protected from light.

Reagent VI: 10 mL×1 bottle. Storage at 4℃, protected from light.

Standard: powder ×1 bottle, add 5 mL of Reagent I before use. 1 mg/mL triglyceride standard solution, storage at 4℃.

Product Description:

Triglyceride(TG) is a fat molecule formed by long-chain fatty acids and glycerol, which is not only the main component of cell membrane, but also an important respiratory substrate. The TG is extracted with isopropyl alcohol, then hydrolysis to glycerol and fatty acids after saponification of TG by KOH. Glycerol is oxidized by periodic acid to form formaldehyde. Condensation of formaldehyde and acetylacetone to form yellow components in presence of chloride ions. The yellow component has a characteristic absorption at 420 nm and proportional to the TG content.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, micro glass cuvette/96 well flat-bottom plate, n-heptane, isopropyl alcohol, water bath, adjustable pipette, distilled water and 125 mL of empty bottle.

Procedure:

I. Sample preparation:

1) Tissue:

Ice-bath homogenate is conducted according to the ratio of tissue mass (g): Reagent I volume (mL) = 1: 5~10 (it is suggested to take about 0.1 g of tissue and add 1 mL of Reagent I). Centrifuge at 8000 g for 10 minutes at 4℃, supernatant is used for test.

2) Bacteria:

Collect 5 million cells or bacteria into the centrifuge tube, then discard the supernatant, final add 1 mL of Reagent I. Splitting bacteria or cell with ultrasonic for 1 minute (power 20%, work time 2s，interval 1s).

Centrifuge at 8000 g for 10 minutes at 4℃, supernatant is used for test.

3) Serum: Detect directly.

II. Procedure:

1. Preheat Spectrophotometer for 30 minutes, adjust the wavelength to 420 nm, set zero with distilled water.
2. Preheat water bath to 65℃.

Reagent name (μL)	Blank tube (A_B)	Standard tube(A_S)	Test tube(A_T)
Distilled water	40	-	-
Standard solution	-	40	-
TG test solution	-	-	40
Reagent I	125	125	125
Reagent II	25	25	25
Mix thoroughly after adding Reagent I, add Reagent II, shake strongly for 30 s, stand several minutes. After layering, 15 μL of the upper layer solution is taken and put it into a new EP tube.			

3. Detect TG content:

Reagent name (μL)	Blank tube (A_B)	Standard tube(A_S)	Test tube(A_T)
Upper layer solution	15	15	15
Reagent III (uL)	50	50	50
Reagent IV (uL)	15	15	15
Mix thoroughly, water bath at 65℃ for 3 minutes.			
Reagent V (uL)	50	50	50
Reagent VI (uL)	50	50	50
Mix thoroughly, water bath at 65℃ for 3 minutes.			

After cooling, transfer liquid from the EP tube to micro glass cuvette/96 well flat-bottom plate, and determine the absorbance at 420 nm.

Note: Blank tube and standard tube only need to be measured once.

III. Calculation:

1 Serum:

$TG(mg/dL)= C\times(A_T- A_B)\div(A_S- A_B)\times100 =(A_T- A_B)\div(A_S- A_B)\times100$

2 Tissue:

Protein concentration:

$TG(mg/mg\ prot)= C\times V\times(A_T- A_B)\div(A_S- A_B) \div(Cpr\times V)=(A_T- A_B)\div(A_S- A_B) \div Cpr$

Sample weight:

$TG(mg/g) = C\times V\times(A_T- A_B)\div(A_S- A_B) \div W=(A_T- A_B)\div(A_S- A_B) \div W$

3 Bacteria or cell:

$TG(U/10^4\ cell) = C\times(A_T- A_B)\div(A_S- A_B) \div D=(A_T- A_B)\div(A_S- A_B) \div D$

1dL =100 mL;

V: The volume of reagent1, 1 mL;
C: Standard concentration, 1 mg/ mL;
Cpr: Sample protein concentration (mg/mL);
W: Sample weight(g);
D: Density of bacteria or cell, 10^4 cell/mL.

Note:

1. There are volatile substances in the kit. Gloves and masks should be worn during the experiment. The reagent bottle cap should be closed in time after opening.
2. After the addition of Reagent II, it is necessary to repeatedly and violently vibrate, so that the triglyceride in the test solution can be fully extracted, and the oscillation amplitude, time, repeated times and waiting for stratification time should be consistent.
3. In order to ensure the repeatability of the test, the cooling time after each water bath should be unified.
4. If the OD value of the test tube is greater than 1.5, it is recommended to dilute the sample with Reagent I properly before testing, and multiply it by the corresponding dilution multiple during calculation.

Recent Products Citations：

[1] Wei Hu,Rui Wei,Liyue Wang,et al. Correlations of MMP-1, MMP-3,and MMP-12 with the degree of atherosclerosis, plaque stability and cardiovascular and cerebrovascular events. Experimental and Therapeutic Medicine. 2018;(IF1.448)

[2] Jieyong Xing,Yanshao Liu,Tao Chen. Correlations of chemokine CXCL16 and TNF-α with coronary atherosclerotic heart disease. Experimental and Therapeutic Medicine. November 2017;(IF1.448)

[3] Zhenbin Xu,Xizhuang Bai. Strontium ranelate-induced anti-adipocytic effects are involved in negative regulation of autophagy in rat bone marrow mesenchymal stem cells. International Orthopaedics. October 2018;(IF2.384)

[4] Chu X Y, Yang S Z, Zhu M Q, et al. Isorhapontigenin Improves Diabetes in Mice via Regulating the Activity and Stability of PPARγ in Adipocytes[J]. Journal of Agricultural and Food Chemistry, 2020, 68(13): 3976-3985.

[5] Li W, Li Y, Zhao Y, et al. The protective effects of aloperine against ox-LDL-induced endothelial dysfunction and inflammation in HUVECs[J]. Artificial Cells, Nanomedicine, and Biotechnology, 2020, 48(1): 107-115.

References：

[1] Fletcher M J. A colorimetric method for estimating serum triglycerides[J]. Clinica Chimica Acta, 1968, 22(3): 393-397.

[2] Hercules D M, Sheehan T L. Chemiluminescent determination of serum glycerol and triglycerides[J]. Analytical chemistry, 1978, 50(1): 22-25.

Related Products：

NA0733/NA0491 Free Cholestenone(FC) Content Assay Kit

NA0808/NA0566 Acetaldehyde Dehydrogenase(ALDH) Activity Assay Kit

NA0834/NA0592 Acetyl CoA carboxylase(ACC) Activity Assay Kit

NA0727/NA0485 Total Cholesterol(TC) Content Assay Kit

Technical Specifications:

The detection limit: 0.0372 mg/mL

Linear range: 0.0625-3 mg/mL